



Where you deliver water and how is a powerful tool.

Philip Schmuck

quoted in *Conservation Foundation Letter*, May 1979

Forum

Yeast Gene and Cadmium

For almost 20 years, the field of phytoremediation has explored ways to use plants to extract toxic materials from soil. Recent advances may offer the promise of economical alternatives to the traditional, labor-intensive phytoremediation technique of removing and incinerating contaminated earth. Most researchers in the field are focused on identifying and refining naturally occurring plants that concentrate pollutants, typically heavy metals, in their cells. But recent work with fission yeast may lead to another potential route—albeit a long one—through which genetic engineering would result in new strains of high-yield, metal-accumulating plants. This work also may be useful for identifying plants that can better tolerate and store metals, says David Ow, a molecular geneticist at the Plant Gene Expression Center of the U.S. Department of Agriculture's Agricultural Research Service in Berkeley, California, and the University of California at Berkeley.

Ow's group has identified the mechanism and the gene responsible for the capability of a fission yeast, *Schizosaccharomyces pombe*, to move the heavy metal cadmium across its cell membranes. For some time, scientists have known that *S. pombe* behaves much like certain plants and fungi that have developed coping strategies for surviving in metal-rich environments fatal to most organisms. *S. pombe* responds to cadmium by producing small peptides called phytochelatins that are rich in the amino acid cysteine. These phytochelatins bond with the cadmium ions, which allows them to transport the ions across cell membranes and into the yeast's vacuole, where the metal accumulates. Once in the vacuole, the ion-peptide complex stabilizes as a crystallite. This "cellular trashbag" can swell with metals until cadmium accounts for as much as 90% of the cell's volume, Ow says.

To isolate the gene responsible for this behavior, Ow compared normal *S. pombe* to cadmium-sensitive *S. pombe* mutants. The mutant yeast failed to chelate, it turned out, because they lack a single gene—dubbed *HMT1*, for "heavy metal tolerance"—which codes for the critical peptide. Now that Ow knows how *S. pombe* triggers the production of the phytochelatins, he is investigating exploiting this cellular "pump" through two

angles. One way may be to bioengineer the yeast gene into plants that already tolerate heavy metals well enough to survive in polluted soil, but that aren't so-called "hyperaccumulators" of metals. Another way may be to identify an equivalent gene already present in metal-tolerant plants.

"The neat thing about it in yeast is that if you overproduce this [peptide], you get an increased rate of transport," Ow explains. "[The yeast] also ends up accumulating more metals in the vacuole, while at the same time becoming more resistant to cadmium. So the hunt is now on to look for a similar protein in plants. Assuming that the whole system is analogous, there should be a similar protein. If we can clone it out and overproduce it so that the plant makes lots more of these proteins, we may be able to pump more metals into the vacuole. Therefore, the plant can pick up more metals as a whole."

Genetically engineering such plants is important, Ow says, because although unaltered hyperaccumulators concentrate heavy metals at high levels (up to 1% of dry weight for cadmium and 5% for zinc and nickel), the plants themselves are too small to extract significant quantities of pollutants.

Other researchers, however, aren't so sure. "I can do more phytoremediation with natural metal-hyperaccumulator plants than they have any hope of doing with these plants that don't have the genetic capability," says research agronomist Rufus Chaney of the U.S. Department of Agriculture's environmental chemistry lab in Beltsville, Maryland. Engineering plants to collect more metals is not useful if those metals collect in the roots, which cannot be harvested practically, Chaney says. Furthermore, he continues, it's not necessarily a simple matter to switch the metal collection site from the plant's roots to its shoots. Finally, he says, "Obtaining expression of this gene at high levels in the membrane of xylem parenchyma cells to pump metals from the cytoplasm into the xylem would [require] further novel bioengineering . . . to make this gene relevant to phytoremediation rather than [merely] trapping cadmium in the roots."

According to Ilya Raskin, a Rutgers University molecular biologist, current phytoremediation techniques don't depend heavily on the process Ow has identified. Instead, workers treat contaminated soil to

dissolve metals and produce a soil solution that metal-resistant plants can draw in through their roots, concentrating metals that are then harvested. Says Raskin, "It's a collection system and it doesn't rely on intricate cellular processes of metal transport." Still, he says, "Only history will tell whether [the cadmium research] will . . . have any relevance to phytoremediation."

Changes to Classifying Carcinogens

Everyone knows that saccharin causes cancer, right? Wrong, according to the National Toxicology Program (NTP), which is expected to delist the chemical from the ninth *Report on Carcinogens*, where it has been classified as "Reasonably Anticipated to Be a Human Carcinogen" since 1981. In the same review, to be held 30–31 October 1997 at the NIEHS, the NTP Board of Scientific Counselors' *Report on Carcinogens* Subcommittee will also examine the toxicity data on 13 other substances, and will expand the traditional scope of substances eligible for consideration for listing in the *Report on Carcinogens* to include chemical mixtures (such as in smokeless tobacco products) and exposure circumstances (such as UV radiation).

The NTP is required by law to prepare a report that contains a list of all substances that are either known to be human carcinogens or may reasonably be anticipated to be human carcinogens and to which a significant number of persons residing in the United States are exposed. The law also states that these reports should provide available information on the nature of exposure, the estimated number of persons exposed, and the extent to which the implementation of federal regulations decreases the risk to public health from exposures to these chemicals. The eighth volume of this report is nearing completion and is scheduled to be published later this year.

The preparation of the ninth report differs from previous reports in several significant ways. Traditionally, the *Report on Carcinogens*, unlike the *Monographs on the Evaluation of Carcinogenic Risks to Humans* prepared by the International Agency for Research on Cancer (IARC), have not examined and discussed evidence for the carcinogenicity of manufacturing processes and

occupational exposures. This has been due in part to the difficulty of placing into categories human exposures that range from single chemicals to complex mixtures and occupational exposure circumstances. It also stems from the ambiguous language in the mandatory statute that requires the listing of carcinogenic "substances" in the *Report on Carcinogens*.

However, a recent series of events has led the NTP to conclude that it would benefit public health to expand the scope of what is eligible for consideration for listing. First, the question of whether the *Report on Carcinogens* should specifically address manufacturing processes and occupational exposures was considered during the recent public evaluation of the draft report of the eighth *Report on Carcinogens*. The Board of Scientific Counselors and the various ad hoc and federal committees that participated in this evalua-

tion agreed that the *Report on Carcinogens* should include reference to the fact that IARC has examined those manufacturing processes or occupations that have been determined to pose a cancer threat to exposed workers, and that the NTP will also examine them in the future. The committees also felt strongly that any inclusion in the report of complex exposure circumstances should be accompanied by statements indicating that such circumstances may differ in various countries or may change over time.

In addition, the NTP recently requested an opinion from the Department of Health and Human Services general counsel on whether manufacturing processes and occupational exposures were legally eligible for consideration for formal listing in the *Report on Carcinogens*. The opinion of the general counsel was that the NTP has broad latitude to consider exposures to other than single

chemicals in the determination of carcinogenic threats to humans.

Finally, increased technological capabilities in exposure assessment and epidemiologic evaluations means that the best scientific criteria can be brought to bear on questions of carcinogenicity. Nominations for listing in the ninth *Report on Carcinogens* reflect the NTP's new attitude, and summary data are provided for agents, substances, mixtures, and exposure circumstances. For example, in addition to such chemicals as cadmium (which is used in batteries and alloys), 1,3-butadiene (which is used in the manufacture of rubber), and the drug phenolphthalein (which is used in laxatives), nominations also include inorganic sulfuric acid mists (used in metal smelting and manufacturing), UV radiation from both solar and artificial sources, and both smokeless tobacco and tobacco smoking.

In addition to the changes in the

Summary of Substances, Mixtures, or Exposure Circumstances Nominated for Consideration of Listing in or Delisting from the Ninth *Report on Carcinogens*

Substance or Exposure Circumstance	Primary Uses or Exposures	Nominated for
Benzidine-based dyes (as a class)	Benzidine-based dyes are used primarily for dyeing textiles, paper, and leather products; more than 250 benzidine-based dyes have been reported by the Society of Dyers and Colorists	Listing as "Known to Be a Human Carcinogen"
1,3-Butadiene	Used primarily as a chemical intermediate and polymer component in the manufacture of synthetic rubber	Upgrading current listing to "Known to Be a Human Carcinogen"
Cadmium and cadmium compounds	Used in batteries, coating and plating, plastic and synthetic products, and alloys	Upgrading current listing to "Known to Be a Human Carcinogen"
Chloroprene	Used as a monomer for neoprene elastomers, industrial rubber products, and as a component of adhesives in food packaging	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
Phenolphthalein	Used as a laboratory reagent and acid-base indicator, and as a cathartic drug in over-the-counter laxative prescriptions	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
Saccharin	Used primarily as a nonnutritive sweetening agent	Delisting from the <i>Report on Carcinogens</i>
Smokeless tobacco	Oral use of smokeless tobacco products	Listing as "Known to Be a Human Carcinogen"
Sulfuric acid mists	Used in the manufacture of fertilizers, rayon and other fibers, pigments and colors, explosives, plastics, coal-tar products such as dyes and drugs, storage batteries, synthetic detergents, natural and synthetic rubber, pulp, and paper, cellophane, and catalysts; also used in petroleum refining, pickling of iron, steel, and other metals, and ore concentration	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
Tamoxifen	Used as an anti-estrogen drug and in the palliative treatment of breast cancer	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
2,3,7,8-Tetrachlorodibenzo- <i>p</i> -dioxin	Used only as a research chemical; potential exposure from municipal incinerators, dump sites, and contaminated soil	Upgrading current listing to "Known to Be a Human Carcinogen"
Tetrafluoroethylene	Used in the production of polytetrafluoroethylene (Teflon) and other polymers; has also been used as a propellant for food product aerosols	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
Tobacco smoke	Inhalation of tobacco smoke	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
Trichloroethylene	Used as an industrial solvent for vapor degreasing and cold cleaning of fabricated metal parts; has also been used as a carrier solvent for the active ingredients of insecticides and fungicides; as a solvent for waxes, fats, resins, and oils, as an anesthetic for medical and dental use, and as an extractant for spice oleoresins and for caffeine from coffee	Listing as "Reasonably Anticipated to Be a Human Carcinogen"
UV radiation	Solar and artificial sources of ultraviolet radiation	Listing as "Reasonably Anticipated to Be a Human Carcinogen"



Problems at the pump? Findings of a recent federal report on oxygenated fuels do little to dispel debate over the health effects of MTBE.

substance of the *Report on Carcinogens*, the process by which the report is prepared and reviewed is also being broadened to promote more public input.

Fueling the Debate

Touted as a way to protect public health by decreasing carbon monoxide (CO) emissions from automobiles, oxygenated fuels containing methyl tertiary butyl ether (MTBE) have been blamed anecdotally for causing headaches, dizziness, eye irritation, burning of the nose and throat, disorientation, and nausea in motorists. In addition, studies have shown that MTBE can cause cancer in rats and mice, though it appears to be a less potent carcinogen than many of the other chemicals found in gasoline and exhaust. Most recently, wells that supply drinking water to Santa Monica, California, were shut down due to high levels of MTBE contamination.

Concerns about the health risks of MTBE, combined with doubts about its ability to significantly lower CO emissions, have caused many to question the usefulness of the chemical as a fuel additive. However, proponents point to decreases in nationwide CO levels as evidence that the 1990 amendments to the Clean Air Act, which sparked the widespread use of MTBE by requiring the use of oxygenated fuels in areas with high CO levels, have led to greatly improved air quality.

Disagreement over the safety and practicality of using MTBE in fuels spurred the EPA to request that the White House National Science and Technology Council review studies done on oxygenated fuels and compile the *Interagency Assessment of Oxygenated Fuels*, which was published in

June 1997. The report considers the effects that using MTBE-treated fuel may have on air quality, water quality, fuel economy, engine performance, and human health.

However, the report does not make any policy recommendations about the future of MTBE as a fuel additive. Ronald Melnick, a toxicologist with the NIEHS who contributed to the interagency report, says, "As far as the total picture of exposure and health effects of oxygenated fuels versus nonoxygenated fuels, there is just not enough evidence right now to draw any definitive conclusion on comparative cancer risk."

As for the effectiveness of MTBE, the authors of the report did find evidence that the chemical, under certain conditions, can decrease CO levels, but they also point out that its performance has not met expectations. "The effectiveness of MTBE was based on models that predicted a 25% decline in CO emissions, which we just have not seen," says Melnick. "CO emissions are decreasing, but it's incorrect to say that this is only a result of using oxygenated fuels. A large part of the decrease is due to improved emission control technology." The report notes that MTBE also cuts other harmful emissions such as benzene and possibly 1,3-butadiene, which is 100 times more carcinogenic than the additive, but that it increases emissions of aldehydes such as the metabolite formaldehyde, which the EPA and the IARC have labeled a genotoxin and probable human carcinogen.

How such changes in emissions and in the composition of fuel vapors will affect motorists is still uncertain, the report concludes. "Complaints have been raised and have not been dismissed about acute health effects like dizziness, headaches, [and] nausea," says Melnick. "There seems to be some consistency

in these reports." But the authors of the interagency report could not find enough evidence to support the contention that MTBE, as used in the winter oxygenated fuels program, is significantly increasing these effects at levels over background levels.

According to the report, the interagency assessment "found that chronic noncancer health effects (neurological, developmental, or reproductive) would not likely occur at environmental or occupational exposures to MTBE." However, inhalation of MTBE has been shown to cause cancer in multiple organ sites in rats and mice. "The EPA is working on a health advisory on MTBE that will be issued in the near future," says Robert Hitzig, the technical lead for the EPA's Office of Underground Storage Tanks. "But it's uncertain now whether [MTBE] will be classified as a possible human carcinogen, a probable human carcinogen, or what its classification will be." However, the interagency report points out that the cancer risk to humans posed by MTBE is similar or slightly less than that posed by untreated gasoline vapors.

Though the health effects of MTBE ingestion are less understood than the effects of inhalation, the appearance of the additive in drinking water across the nation has caused concern over this route of exposure. "MTBE absorbs weakly in soil and not very biodegradable," explains Melnick, "so when there are leakages from underground gasoline storage tanks, it travels further in the ground water and persists for long periods of time." Also, MTBE that enters the atmosphere through exhaust and evaporation can fall to earth and flow into surface water reservoirs with precipitation. Recent studies found MTBE in 7% of urban storm water samples and in 5% of well water samples from across the United States. "The health hazards of MTBE in water are debatable," says Hitzig, "but it's not debatable that there are aesthetic problems with it. MTBE has a very low taste and smell threshold, so that if it's in people's water, they probably know it."

It is also unknown if the presence of MTBE in water is taking a toll on aquatic life. Studies so far have focused only on water used for human consumption, but the persistence of the chemical in the environment makes it of particular concern. Also, notes Hitzig, "There's no way of telling how much MTBE has leaked into groundwater. There have been over 300,000 confirmed releases of petroleum from underground storage tanks since 1988, but we don't know what percentage of them had MTBE in them."

MTBE, which is derived from methanol, is now the most widely used oxygenate in the United States. From 1984 to 1995, production has increased by about 26% annually, with 8 billion kg produced in 1995.